Docket Number: 1328-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	Examiner: Amanda M. SHAW
	)	
PADGETT et al.	)	Group Art Unit: 1634
	)	
Serial No.: 10/684,134	)	
	)	I hereby certify that this correspondence
	)	is being transmitted to the USPTO
Filed: October 10, 2003	)	via EFS-Web on November 24, 2008
	)	by /Wayne Fitzmaurice/
For: Polynucleotide Sequence Variants	)	Wayne P. Fitzmaurice
	)	Reg. No. 58,274

DECLARATION UNDER RULE 132

Padgett Declaration 3

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Hal S. Padgett, do hereby declare and state:

1. I received a B.S. in Life Sciences in 1988 from the University of Missouri at Rolla and a Ph.D. in Molecular Microbiology and Microbial Pathogenesis in 1996 from Washington University in Saint Louis, Missouri. I joined Large Scale Biology Corporation in 1998. For nine years my responsibilities involved viral vector development and molecular evolution programs at Large Scale Biology Corporation. Since 2007 I have been the Chief Scientist and General Manager of Novici Biotech, LLC.

2. I am a named inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled POLYNUCLEOTIDE SEQUENCE VARIANTS.

3. This Declaration provides additional details from experiments performed in Example 15 in which a number of gene pairs having various percentages of sequence identity to one another were GRAMMR shuffled. In the Example provided in the specification, the sequences of several typical shuffled clones were provided to exemplify the degree of shuffling that was obtained from these different gene pairs. In this declaration, we include data from those experiments to quantitate the overall percentages of shuffled clones in the resulting libraries. We also describe the frequency of sequence crossovers (resulting from information transfer events) within the population of shuffled clones obtained in those experiments.

Four gene pairs having homology scores of 54%, 65%, 66%, and 75% (as determined by alignment by ClustalX using a gap opening penalty of 10 and a gap extension penalty of 2) were shuffled as described in Example 15. For each of the resulting libraries, between 33 and 77 individual clones were randomly selected and sequenced across their approximately 800 base pair length. For each library, the percentage of shuffled clones (clones bearing at least one crossover) was determined and recorded in Table 1. The overall number of crossovers within the pool of shuffled clones was also tallied to calculate the average number of crossovers per kilobase of sequence in the population of shuffled clones (Table 1).

Percent Identity between parent genes	Number of progeny clones sequenced	Number of shuffled progeny (and % of total)	Total number of crossovers in the shuffled clones	Average number of crossovers per kb of shuffled gene
75%	77	34 (44%)	287	10.6
66%	67	22 (33%)	135	7.7
65%	33	9 (27%)	46	6.4
54%	44	9 (20%)	30	4.2

Table 1. Analysis of shuffled clone libraries resulting from GRAMMR shuffling of gene pairs with various levels of sequence identity. Listed in the first column are the percent identity scores for the various parental gene pairs. The second column shows the numbers of progeny clones that were randomly selected for each analysis. In the third column are the numbers of shuffled clones and their proportion of the total, by percentage, for each library. The fourth and fifth columns list the total number of crossovers present in the set of shuffled clones and the frequency of crossovers per kilobase of shuffled gene sequence, respectively.

There were two objectives in performing the following set of experiments. One objective was to determine the percentage of shuffled versus unshuffled parental clones obtained from GRAMMR shuffled gene libraries constructed from parent gene pairs with various levels of sequence identity to one another. The other objective was to determine the average number of gene crossovers (resulting from information transfer events) present in the population of shuffled clones from each of these parental gene pairings.

From these experiments, we found a correlation between the DNA identity score of the parent genes and the percentage of shuffled clones in the resulting GRAMMR shuffled libraries. In general, the higher the homology score, the higher the percentage of shuffled clones. Secondly, we found a correlation between the DNA identity score of the parent genes and the frequency of crossovers observed in the population of shuffled clones. Genes having higher levels of identity to one another tended to have higher frequencies of crossovers per shuffled clone. In a given experiment, small variations in experimental conditions may affect the outcome such that shuffled clone percentages and crossover frequencies may differ somewhat from what is reported here.

These observations are relevant to Claims 1, 2, 4, 5, and 7-12 of the application.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information are believed to be true, and that the statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, and patent issuing thereon, and patent to which this verified statement is directed.

November 21, 2008
Date

Hal S. Padgett, Ph.D.